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Control of Blood Volume following Hypovolemic Challenge in Vertebrates: Transcapillary *Versus* Lymphatic Mechanisms

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Abstract

Anurans have an exceptional capacity for maintaining vascular volume compared with other groups of vertebrates. They can mobilize interstitial fluids via lymphatic return at rates that are ten-fold higher than mammals. This extraordinary capacity is the result of coordination of specialized skeletal muscles and pulmonary ventilation that vary volume and pressure of subcutaneous lymph sacs, thus moving lymph to dorsally located lymph hearts that return lymph to the vascular space. Variation in the capacity to mobilize lymph within anurans varies with the degree of terrestriality, development of skeletal muscles, lung volume and lung compliance, and lymph heart pressure development. This ability enable anurans, which have the highest rates of evaporative water loss among terrestrial vertebrates, to withstand levels of dehydration far exceeding that of other vertebrates, and to successfully occupy virtually all terrestrial environments during their evolution. Maintenance of vascular fluid volume for all vertebrates can be achieved primarily by moving fluid from the interstitial space to the vascular space by transcapillary uptake and mobilization of interstitial (lymphatic) fluid. Transcapillary fluid uptake at the capillary level has been analyzed historically by Krogh and others from a Starling perspective and involves a balance of hydrostatic and oncotic forces. A complete evaluation of blood volume homeostasis also incorporates pressures and compliances of the vascular and interstitial spaces, but has been applied to only a few species. In this review we outline the current understanding of how anurans and other vertebrates maintain blood volume during hypovolemic challenges such as dehydration and hemorrhage which is crucial for maintaining cardiac output.

Introduction

August Krogh (1874-1949) performed ground-breaking work on the regulation of capillaries to supply O_2 to working muscles, for which he was awarded the Nobel prize in physiology (Poole et al. 2021; Kissane et al. 2021), but he also recognized that very little was known about the permeability of the capillary walls (Krogh, 1922). This statement caught the attention of Eugene Landis (1901-1987), a medical student at the University of Pennsylvania, who embarked on a series of studies aimed at understanding the mechanisms of transcapillary fluid flux between the vascular and interstitial spaces (Landis, 1934). The impact of Landis’ work greatly impressed Krogh and, in 1929, Landis obtained a two-year Guggenheim Fellowship to work with Krogh in Copenhagen and with Sir Thomas Lewis (1881-1945) in London to quantify the rate of capillary fluid exchange in humans (Krogh et al. 1932).

Because all vertebrates have in common a closed circulatory system, they share the same physical and physiological constraints on plasma volume homeostasis; these factors include transvascular fluid flux, as well as pressure and compliance of the vascular and interstitial compartments. Maintaining adequate blood volume is crucial to ensure that cardiac output is not compromised across a range of activity states (Joyce and Wang, 2021). Confronted with blood volume loss, vertebrates can either restore vascular volume from the interstitial fluid volume (transcapillary uptake) or mobilization of lymphatic return to the circulation.

Transcapillary fluid flux, and the balance of forces across the capillary that dictate the overall direction of fluid movement, was first described by Ernest Starling (Starling 1896); however, Landis was the first to experimentally test Starling’s hypotheses through direct measurement of capillary pressures and transcapillary fluxes in anurans and other animals (Landis, 1934). Because capillary dynamics vary among tissues, more recent work recognized the need to describe transvascular fluid fluxes at the whole animal level by integrating additional information such as the role of vascular and interstitial compliances. This led to the analysis by Tanaka (1979) that incorporates these additional concepts into an overall analysis of transvascular fluid flux for mammals (dog), but is readily applicable to all vertebrates.

Comparative capabilities for maintaining vascular volume

The volume of blood contained in the vascular system is composed of both cells and plasma. The fraction of blood composed of cells is defined as the hematocrit. Because blood viscosity increases exponentially with hematocrit, loss of plasma volume creates a dual threat to decreasing cardiac output via a reduction in venous return to the heart and an increase in power required to pump the blood owing to the increased viscosity. Consequently, the capacity to maintain both plasma volume and cardiac output during dehydrational challenge by mobilizing interstitial fluid is an important adaptation in terrestrial environments (Hillman 2018). The relative ability of anuran amphibians (frog and toad) compared with a representative mammal (human) illustrates the extent to which anurans are capable of maintaining plasma volume in the face of dehydrational and hemorrhagic stresses (Fig. 1).

Transcapillary Mechanisms

Transcapillary fluid flux represents a balance of forces between hydrostatic and oncotic (capillary and interstitial) pressures, thus the magnitudes of these forces dictates whether fluid flux is outward (i.e. plasma to interstitial) or inward (i.e. interstitial to plasma). Starling (1896) recognized the importance of plasma oncotic force to balance the hydrostatic force exerted by capillary hydrostatic pressure and the overall balance of forces from a Starling (1896) analysis is provided:

$$J_v = F_c\,[(P_c - P_i) - \sigma\,(\Pi_{pl} - \Pi_i)], \qquad [1]$$

where J_v is volume flux, F_c is the filtration coefficient, P_c and P_i are pressures in the capillary and interstitial compartments, respectively, and Π_{pl} and Π_I are the oncotic pressures in the plasma and interstitial compartments, respectively, and σ is the protein reflection coefficient. A decrease in capillary pressure below the oncotic pressure would lead to fluid uptake from the interstitial fluid around the capillary, a situation that occurs with fluid loss associated with hemorrhage (Michel et al. 2020). In mammals, capillary plasma protein permeability is low ($\sigma \geq 0.9$), thus the small fluid efflux can easily be balanced by lymphatic return (Michel et al. 2020). However, when capillary protein permeability is high (i.e. $\sigma < 0.9$), and there is little difference between plasma and interstitial protein colloid concentrations, the Starling model has limited utility. Indeed, Landis (1934) concluded that…*if large amounts of protein are lost in the capillary filtrate the concept of balance between capillary pressure and the colloid osmotic* *pressure of the blood becomes untenable*. This is the normal situation for fish and amphibians (Olson et al. 2003; Hillman et al. 2010b), but is unknown for reptiles and birds.

To account for the limitations of the Starling model, Tanaka (1979) incorporated the physical characteristics of volume and compliance of the vascular and interstitium:

$$J_v = F_c\,[(V_{vas}/C_{vas}) - (V_i/C_i)], \qquad [2]$$

where J_v is volume flux, F_c is the whole-body filtration coefficient, V is the volume of blood in the vascular (V_{vas}) or the interstitial compartment (V_i), and C is the compliance of the vascular (C_{vas}) or interstitial compartment (C_i). In essence, the term (V_{vas}/C_{vas}) in Equation 2 represents efflux and the term (V_i/C_i) represents influx. Because compliance is Volume/Pressure, Equation 2 can be reduced to:

$$J_v = F_c\,(P_{vas} - P_i), \qquad [3]$$

where J_v is the product of F_c and the pressure difference between the vascular and interstitial space. It is clear that compliance plays an important role by determining the pressure in the vascular or interstitial compartment in response to a given volume change. The drawback of this analysis is that compliance characteristics of the vasculature and interstitial space are available for only three representative vertebrates: mammals (Tanaka, 1979), anuran amphibians (Hillman et al. 2010b) and bony fish (Olson et al. 2003).

The degree to which net fluid flux is influenced by variations in vascular and interstitial variables differs considerably among the vertebrates. For example, trout and toad capillaries are highly permeable to fluids and plasma proteins compared with mammalian capillaries (Hargens et al. 1974; Baldwin et al. 1993), reflected by 5-8 fold higher filtration coefficients than for mammals (Fig. 2A). This would suggest that the Starling model has limited predictive value for these groups. In trout, the high capillary permeability is combined with a low interstitial compliance that creates a balance between vascular and interstitial pressures under normal conditions (Fig. 2D). Rapid changes in vascular compliance, through hemorrhage or volume infusion (Olson et al. 2003), produces rapid fluid movement between the vascular and interstitial compartments as predicted by the Tanaka model (Equation 3). In the toad, the high capillary permeability is coupled with an extremely high interstitial compliance relative to fish and mammals (Fig. 2C). The large interstitial compliance creates a situation in anurans where efflux exceeds influx (i.e. V_i/C_i is very small) under all conditions regardless of how any of the vascular variables change (Fig. 2D; Hillman et al. 1987). Interestingly, the dog, which is predicted by the Tanaka model to have a net uptake of fluid under normal conditions, has a net efflux of fluid in a pathophysiological state such as edema (Fig. 2D) which is characterized by a high interstitial compliance similar to the toad. The large interstitial compliance in anurans (and in the edematous state for mammals) ensures that very little increase interstitial pressure develops despite the large volume flux from the vascular to the interstitial space. Neither the Starling model (Equation 1) nor the Tanaka model (Equations 2 and 3) can provide a transcapillary uptake mechanism for the toad, thus high rates of lymph flux are required to maintain plasma homeostasis. A key feature of the anuran system, and unique among vertebrates, is the large interstitial compliance that promotes lymph storage that can be mobilized during dehydration. This lymph storage capability was likely important for the evolutionary success of anurans.

Lymph Mobilization Mechanisms in Anurans

The anuran lymphatic system is composed of a number of large subcutaneous lymph sacs that carry lymph to two pairs of dorsally-located lymph hearts that contract and return lymph to the venous circulation. Landis (1934) recognized that the *combination of highly permeable cutaneous capillaries with lymph hearts and rapid circulation of body fluids represents, therefore, a specialized mechanism, which must be kept in mind in studies of fluid balance in amphibians*. The fascinating aspect of this design is how pressure differences are created to move the lymph to the lymph hearts.

The formation of lymph would necessarily drain to the ventral region of the animal due to gravitational forces, thus a major problem to overcome by anurans is the vertical movement of to the dorsally-located lymph hearts (Fig. 3A; Hillman et al. 2004). We have found there are two distinct, but coordinated, mechanisms for the vertical movement of lymph: 1) specialized skeletal muscles that change the volume of lymph sacs that create a pressure gradient for lymph movement with capillary ultrafiltration; 2) lung expiration that creates a negative pressure in the subvertebral lymph sac that aspirates lymph in a vertical direction, and then communicates directly with the lymph hearts (Fig. 3A; Hillman et al. 2010a; Hedrick et al. 2011). In addition, horizontal movement of lymph in the hind limb is created by differential compliance, and therefore a pressure gradient, relative to mass in each limb segment as predicted by the Tanaka model (Fig. 3A; Hillman et al. 2005).

The specific lymph skeletal muscles involved with lymph movement were determined through anatomical dissections and measurements of muscle activity with electromyography in conjunction with measurements of lymph sac pressures (Drewes et al. 2007; Hedrick et al. 2007). Comparisons between species that are representatives of different environments (aquatic, semi-aquatic and semi-terrestrial) revealed substantial variation, including presence or absence, of the lymph skeletal muscles (Fig. 3C; Hillman et al. 2011). This variation in lymph skeletal musculature is also correlated with lymph flux rates between these species (Fig. 3B; Hillman et al. 2011).

Our hypothesis about the role of these lymph muscles, and the role of lung ventilation (see below), for assisting vertical lymph movement is based on experiments where selective ablation of specific lymph muscles or interference with the ability to maximally inflate/deflate the lungs interferes with overall lymph flux (Hillman et al. 2010a). In addition, visual confirmation of the vertical movement of lymph was revealed using computed tomography by injecting a contrast dye into ventral lymph sacs of conscious toads and showing that the dye reached the lymph hearts (Hedrick et al. 2014).

Negative Feedback Control of Lymphatic Function

Having identified key mechanisms (skeletal muscles and lung ventilation) for overcoming the problem with vertical lymph movement to dorsally-located lymph hearts, a key question is how do these effectors fit into an overall framework of cardiovascular fluid volume homeostasis? Lymph heart frequency is inversely related to mean arterial blood pressure (MAP), but this relationship is abolished after denervation of the primary baroreceptor nerve (recurrent laryngeal nerve) in toads (Fig. 4; Crossley and Hillman, 1999).

Reptiles and birds have strong abilities to mobilize fluid and maintain volume when hypovolemically challenged by hemorrhage or dehydration, and Starling-based transcapillary mechanisms have been invoked to explain these responses (Djojosugito et al. 1968; Lillywhite and Smith, 1981). However, the actual balance of transcapillary forces has not been systematically measured for reptiles or birds, thus the role of Starling forces and lymph mobilization mechanisms in these groups represents a large gap in our understanding of blood volume regulation in vertebrates (Hedrick et al. 2013).

As noted above, lung ventilation also assists in the vertical movement of lymph that suggests that lung ventilation plays a significant role for cardiovascular homeostasis in anurans, in addition to its role in gas exchange. In support of this, respiratory frequency is also inversely related to MAP with a greater change in ventilation occurring with reductions in MAP rather than with hypertension (Fig. 4; Zena et al. 2016). It is important to note that the lymph skeletal muscles contract with each expiration, hence ventilatory frequency and muscle contraction frequency are tightly coupled (Hedrick et al. 2007). Hypotension, through dehydration or hemorrhage, is a greater threat to cardiovascular homeostasis, and represents a strong feedback signal for activation of the effectors of this system: lung ventilation, lymph skeletal muscle pumps and lymph heart activity. The link between MAP and lung ventilation may be a basal vertebrate function that is still retained in a limited degree in mammals (Ohtake and Jennings, 1992).

The importance of lung ventilation to lymph movement is also reflected in inter-family differences in pulmonary characteristics in anurans. More terrestrial, dehydration tolerant species (Bufonidae) having larger and more compliant lungs (Fig. 5; Hedrick et al. 2011; Withers et al. 2014) and is also correlated with differences in lymph flux rates between these groups (Fig. 3). Larger and more compliant lungs would help to mobilize lymph by creating larger volumes for a given change in lung pressure and this would assist vertical lymph movement. Because lung morphology of these species are correlates with terrestriality, rather than metabolism, this role for pulmonary ventilation to mobilize lymph may have been under selection pressure as anurans invaded terrestrial environments.

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Figure Legends

Figure 1. A comparison of the relative capacities of a human, a frog (*Lithobates catesbeianus*) and a toad (*Rhinella marina*) to compensate for dehydration and hemorrhage. **A**. Dehydrational loss of water via evaporation amounting to 10% of initial mass shows that human, as a mammalian example, is severely challenged by this degree of dehydration both in a loss of overall volume and the resulting increase in blood viscosity. The two anurans, a semiaquatic frog and terrestrial toad, handle this challenge better than the mammal and are also interspecifically different: the toad is better able to compensate for the fluid loss with no volume or viscosity effects. **B.** The same pattern holds when these three species are faced with a hemorrhagic volume loss of 3.8% body mass. The toad is able physiologically to maintain maximal blood flow rates while the mammalian cardiac output falls to zero. There are clear interspecific differential capacities to mobilize interstitial fluid to compensate for hypovolemic challenge. Adapted from Hillman (2018).

Figure 2. **A-C**, A comparison of vascular characteristics between trout, toad and dog. **A.** Whole-body filtration coefficients are highest in trout, intermediate in the toad and lowest in dog. **B**. Vascular compliance, reflecting differences primarily in blood volume, is lowest in trout, intermediate in toad and highest in dog. **C**. Comparison of interstitial compliance illustrates the very high C_{ist} in toads compared with fish and mammals. Values from Hillman et al. (2010b). **D**. Sensitivity analysis illustrating the effects of increasing or decreasing by 50% the variables from Equation 2 (Tanaka, 1979) on transcapillary flux for a cane toad (*Rhinella marina*; Hillman et al. 2010b), trout (*Onchorhynchus mykiss*; Olson et al. 2003) and a mammal (domestic dog; Tanaka, 1979). The toad demonstrates a pattern of a net driving force for transcapillary efflux under all conditions in this analysis. The variable primarily responsible for this pattern is the high C_{ist},

created by the large subcutaneous lymph sacs underlying most of the skin. This implies that transcapillary uptake is impossible as a mechanism to explain the anuran capacities to maintain plasma volume with hypovolemic challenge compared to mammals (Fig. 1). Thus, the lymphatic system is required for fluid mobilization due to dehydration or hemorrhage. Under normal conditions for a trout, there is no net driving force, thus variation in the filtration coefficient (F_c) has little or no effect on flux. Increased vascular compliance (C_{vas}; decreased venous tone) or decreased blood volume lead to a net fluid uptake while a net efflux occurs when blood volume (V_b) is increased or venous tone is increased. The trout differs from most other vertebrates in having lower interstitial compliance (C_{ist}) and higher F_c that would work in concert to quickly move fluid between the two compartments with any change in vascular pressure or volume as predicted by the Tanaka (1979) model. This is assumed to be the ancestral vertebrate pattern. The mammalian example (dog) demonstrates a pressure difference for net fluid reabsorption under all conditions even when the variables are increased or decreased by 50%. This changes if the whole body interstitial compliance increases significantly reflecting an edematous condition. The accumulation of fluid in edema creates space in the loose connective tissue anchoring the dermis to the underlying muscle tissue and increases C_{ist} 20-30 times control values. This space is analogous to the subcutaneous lymph sacs of anurans and creates a pressure difference for fluid loss in all situations.

Figure 3. **A**. A schematic representation of the hind limb lymph sacs for the toad, *Rhinella marina*. Lymph moves horizontally from distal (plantar) to proximal (femoral) lymph sacs with increasing compliance, as reflected in the size of the boxes representing these lymph sacs. This creates a distal to proximal pressure gradient (change in relative blue shading) in these lymph sacs. The pathway of lymph movement between the lymph sacs to the dorsally-located lymph hearts (LH) is indicated by arrows. The vertical distance for movement of lymph in this species is approximately 2 cm which is the pressure (0.200 kPa) necessary to move lymph. Figure modified from Hillman et al. (2004). **B**. Lymph flux rates for three species of anurans correlates with terrestriality with lowest flux rates in the aquatic pipid (*Xenopus laevis*) and the highest flux rates in the semi-terrestrial toad (*R. marina*). Adapted from Hillman et al. (2011). **C**. Specialized lymph muscles from an aquatic pipid (*Xenopus laevis*) and semi-aquatic bullfrog (*Lithobates*

catesbeianus) and a semi-terrestrial toad (*Rhinella marina*). See Hillman et al. (2011) for complete descriptions of these muscles and their functions.

Figure 4. Relationship between lung ventilation frequency and lymph heart frequency with mean arterial pressure (MAP) in toads (*Rhinella marina* and *R. schneideri*). Lung ventilation and lymph heart frequency are inversely related to MAP. Lung ventilation increases from baseline values with reductions in MAP that mobilize lymph (see text) and work in concert with increased lymph heart rate. Lymph heart rate is independent of MAP when the primary baroreceptor nerve is cut showing the importance of arterial baroreceptor feedback. Lung ventilation data modified from Zena et al. (2016) and lymph heart data modified from Crossley and Hillman (1999).

Figure 5. Lung volume and lung compliance vary with terrestriality in anurans. **A**. Relationship between lung volume (**A**) and lung compliance (**B**) with body mass among nine species of anurans representing the bufonidae (5 species), ranidae (2 species) and pipidae (2 species). Mean (and s.e.m.) lung volume (**C**) and lung compliance (**D**) values are significantly greater in the more terrestrial species (Bufonidae) and correspond with increased ability to mobilize lymph (see Fig. 3B). Adapted from Hedrick et al. (2011).

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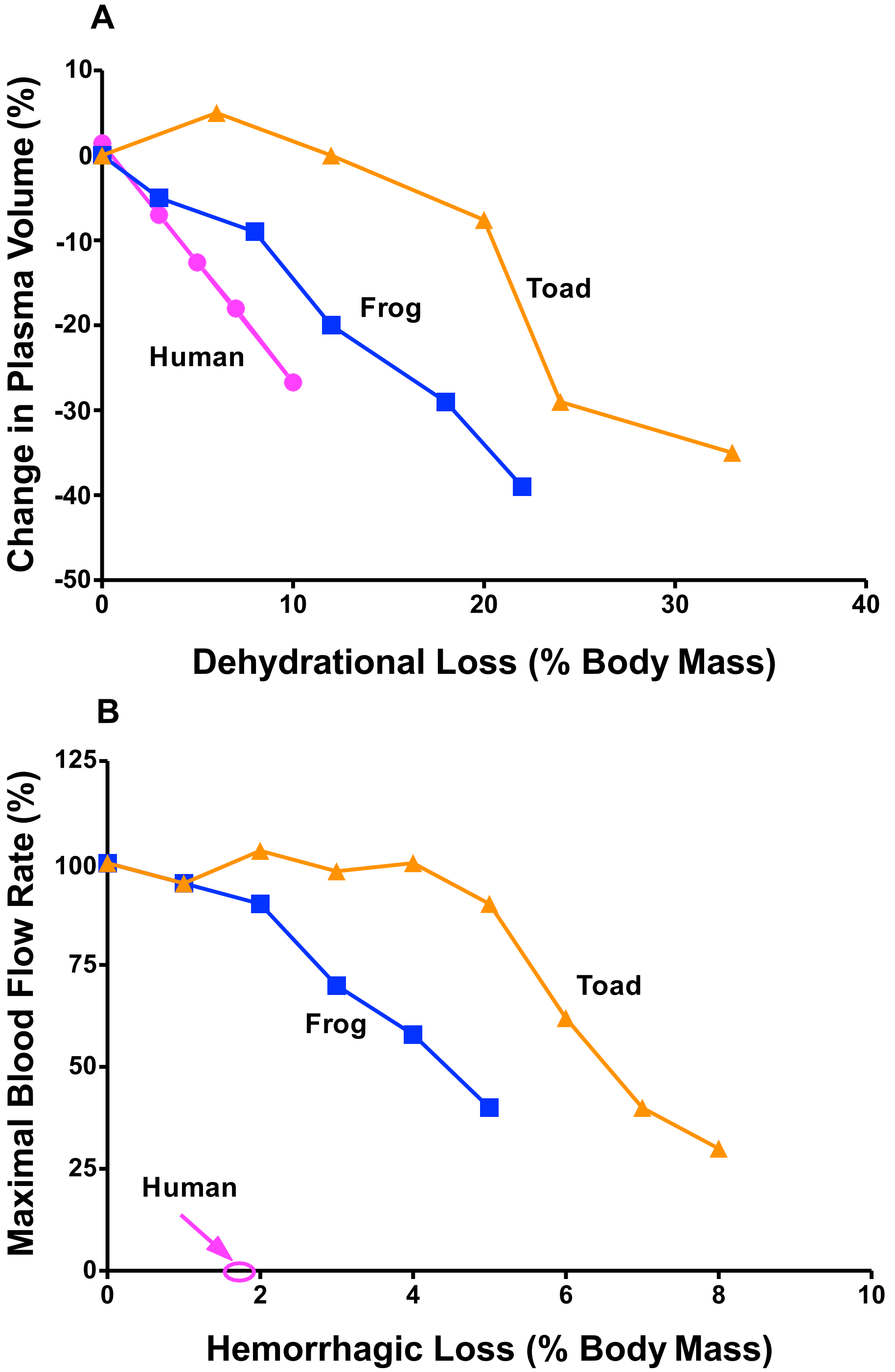
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Change in Plasma Volume (%)
10
0
-10
-20
-30
-40
-50
0
10
20
30
40
Dehydrational Loss (% Body Mass)
Human
Frog
Toad
B
Maximal Blood Flow Rate (%)
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100
75
50
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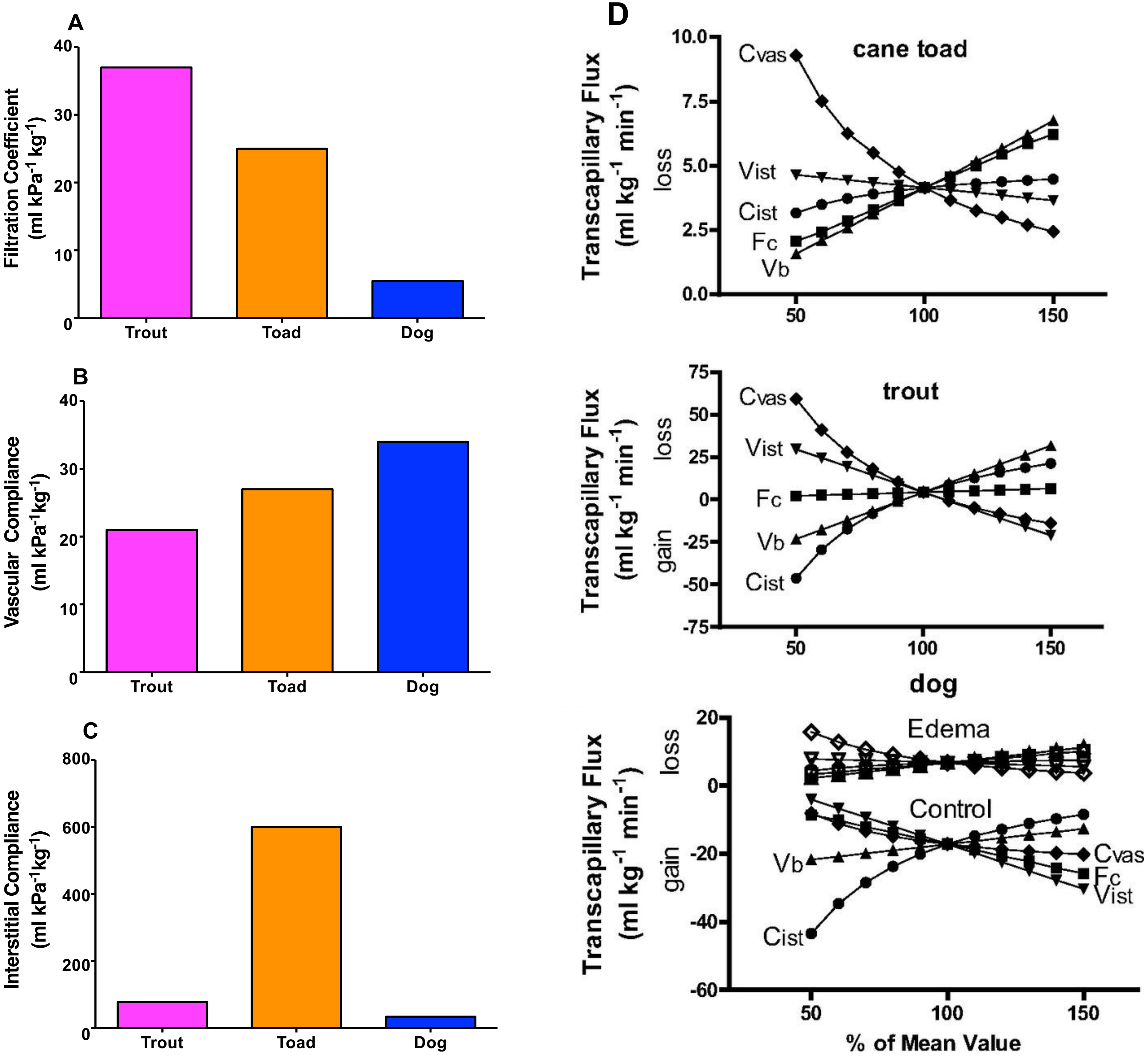
0
0
2
4
6
8
10
Hemorrhagic Loss (% Body Mass)
Human
Frog
Toad

Figure 1


A
Filtration Coefficient (ml kPa^{-1} kg^{-1})
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Trout
Toad
Dog
B
Vascular Compliance (ml kPa^{-1} kg^{-1})
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10
20
30
40
Trout
Toad
Dog
C
Interstitial Compliance (ml kPa^{-1} kg^{-1})
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200
400
600
800
Trout
Toad
Dog
D
cane toad
Transcapillary Flux (ml kg^{-1} min^{-1})
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2.5
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Cvas
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50
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trout
Transcapillary Flux (ml kg^{-1} min^{-1})
loss
gain
-75
-50
-25
0
25
50
75
Cvas
Vist
Fc
Vb
Cist
50
100
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dog
Transcapillary Flux (ml kg^{-1} min^{-1})
loss
gain
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-40
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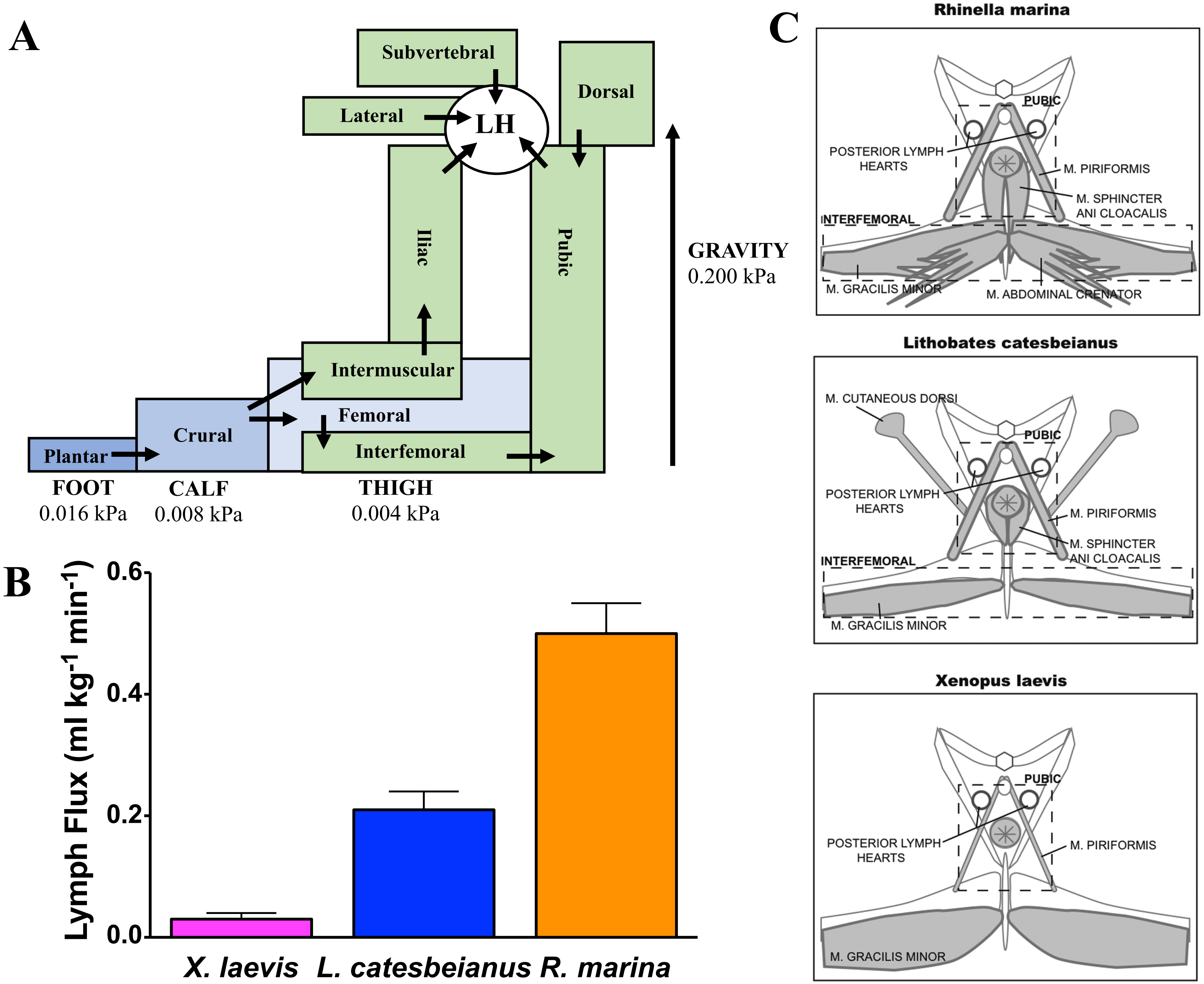
Figure 2

Figure 3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Iliac
Pubic
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Interfemoral
FOOT
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CALF
0.008 kPa
THIGH
0.004 kPa
B
Lymph Flux (ml kg-1 min-1)
0.6
0.4
0.2
0.0
X. laevis
L. catesbeianus
R. marina
C
Rhinella marina
PUBIC
POSTERIOR LYMPH HEARTS
M. PIRIFORMIS
M. SPHINCTER ANI CLOACALIS
INTERFEMORAL
M. GRACILIS MINOR
M. ABDOMINAL CRENATOR
Lithobates catesbeianus
M. CUTANEOUS DORSI
PUBIC
POSTERIOR LYMPH HEARTS
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M. SPHINCTER ANI CLOACALIS
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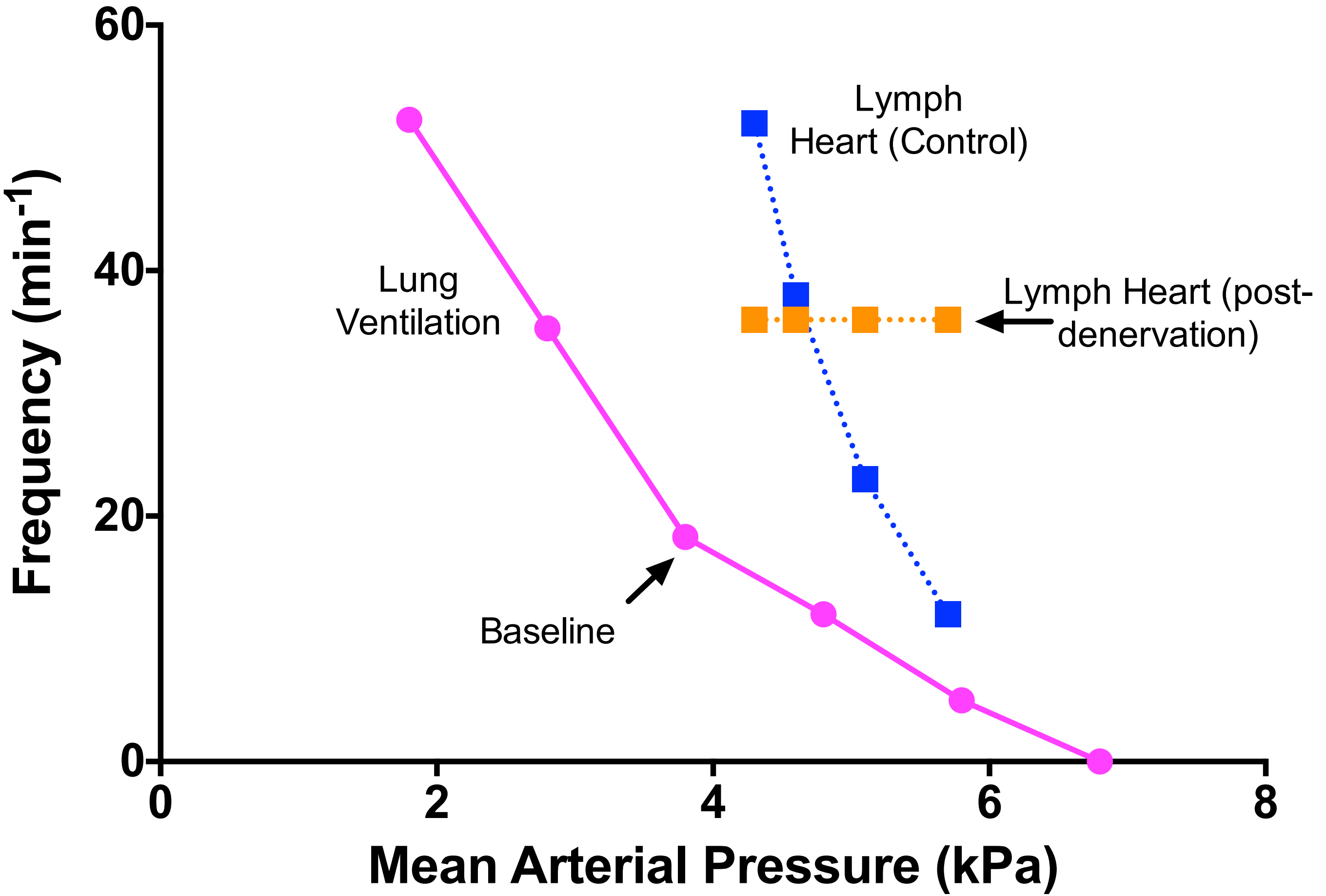
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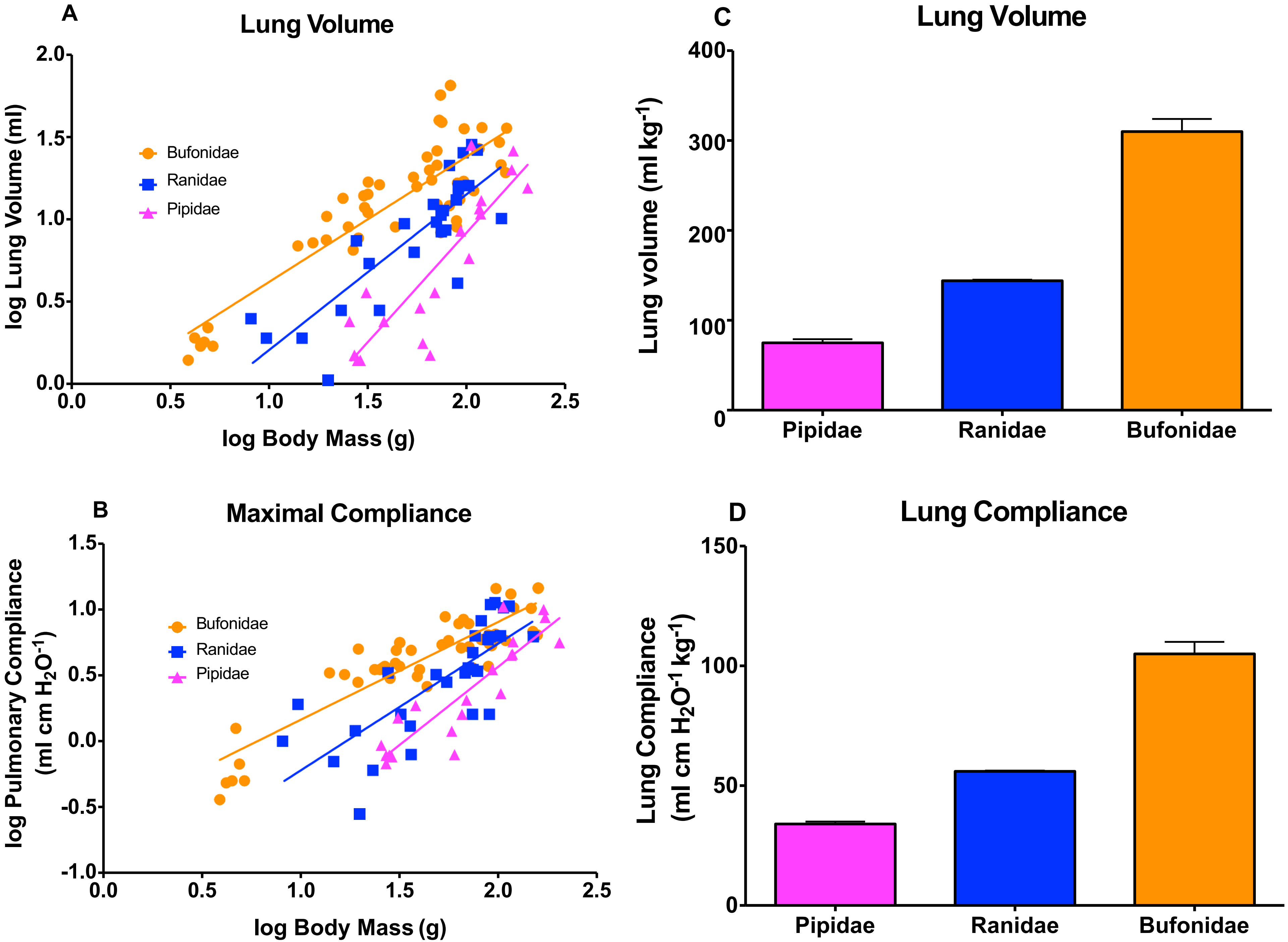
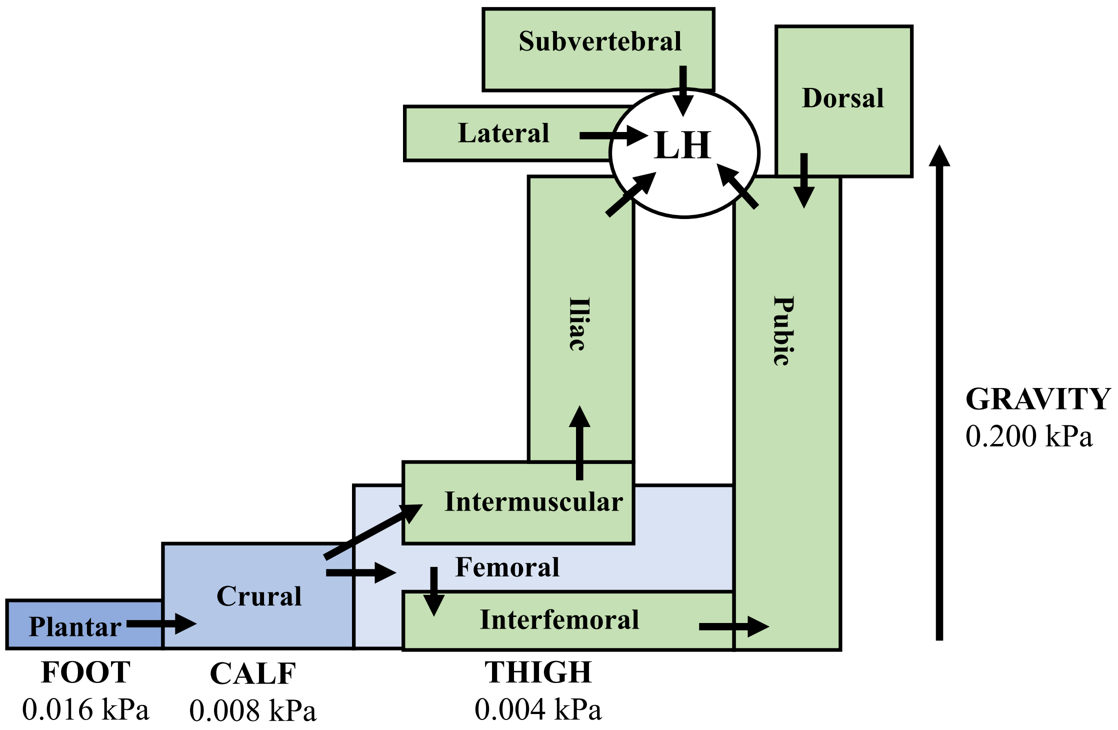
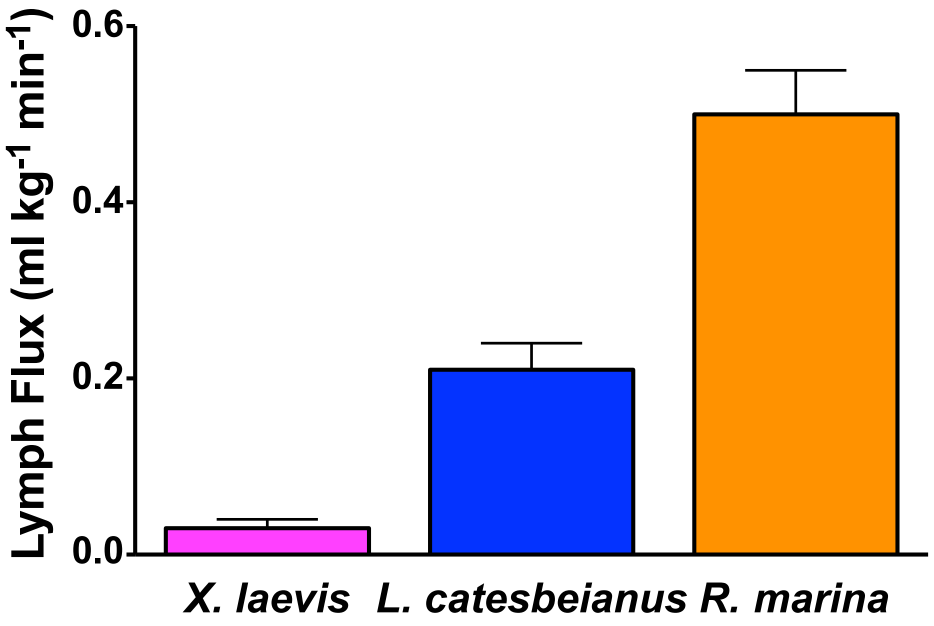
4
6
8
Mean Arterial Pressure (kPa)
Lymph Heart (Control)
Lymph Heart (post-denervation)
Lung Ventilation
Baseline

Figure 5


A
Lung Volume
log Lung Volume (ml)
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5
log Body Mass (g)
Bufonidae
Ranidae
Pipidae
C
Lung Volume
Lung volume (ml kg^{-1})
400
300
200
100
0
Pipidae
Ranidae
Bufonidae
B
Maximal Compliance
log Pulmonary Compliance (ml cm H_2O^{-1})
1.5
1.0
0.5
0.0
-0.5
-1.0
0.0
0.5
1.0
1.5
2.0
2.5
log Body Mass (g)
Bufonidae
Ranidae
Pipidae
D
Lung Compliance
Lung Compliance (ml cm H_2O^{-1} kg^{-1})
150
100
50
0
Pipidae
Ranidae
Bufonidae


Subvertebral
Lateral
LH
Dorsal
Iliac
Pubic
Intermuscular
Femoral
Crural
Interfemoral
Plantar
FOOT
0.016 kPa
CALF
0.008 kPa
THIGH
0.004 kPa
GRAVITY
0.200 kPa


Lymph Flux (ml kg^{-1} min^{-1})
0.0
0.2
0.4
0.6
X. laevis
L. catesbeianus
R. marina